Prevalence and Persistence of Antibiotic Resistance in Food Products

Xinhui Li and Hua H. Wang*

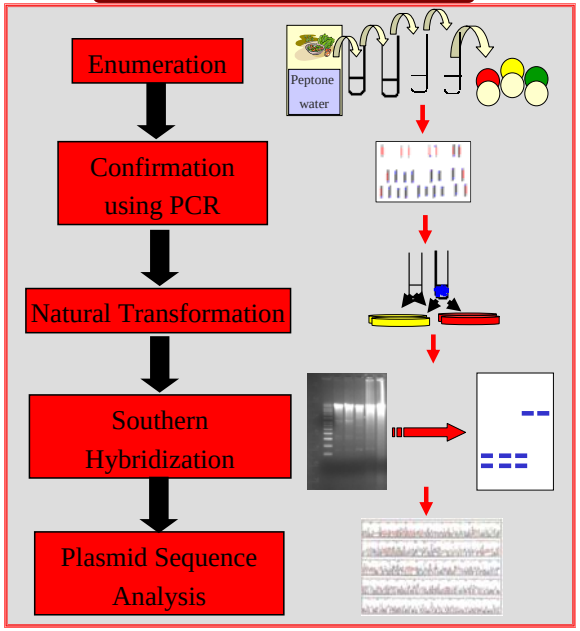
ABSTRACT

Antibiotic resistance (AR) is a global food safety and public health challenge. The objective of this project was to examine the prevalence of AR in selected food items, and the functionality and persistence of the AR determinants. Selected food items including raw food materials as well as deli and processed ready-to-eat items were used in the study. Tetracycline resistant (Tet^r) bacteria were found in raw and deli shrimp, sushi and cheese samples. The *tetS*, *tetL* and *tetM* genes were found in the food isolates by PCR screening. Commensal bacteria including *Carnobacterium sp.*, *Brochothrix sp.* and *Enterococcus sp* were identified to be AR-gene carriers by 16S rRNA gene sequence analysis. The AR genes were found associated with several large plasmids in *Carnobacterium sp.* and *Enterococcus sp.* isolates. A 20-kb plasmid containing both *tetM* and *tetL* was found in an *Enterococcus sp.* isolate from cheese. The plasmid is very stable at the absent of tetracycline, indicating the presence of additional mechanism(s) in maintaining the resistance gene in the stain. The *tet^r* genes from selected food isolates were transmitted to *Sreptococcus mutans UA159* by natural gene transformation and led to acquired resistance in transformants, suggesting the functionality and transferability of the resistance genes from the food isolates. Our results suggest that food has become an important avenue directly transmitting resistant bacteria to humans, and commensal bacteria likely have played an important role in the dissemination of the AR genes. Particularly, our data indicate that antibiotics may not be essential in the maintenance and transmission of these AR genes as believed in the past. These results are of great importance for agriculture and food industry for the development of proper control strategies.

INTRODUCTION

The rapid emergence of ART pathogens is a major threat to public health. AR gene reservoirs have been identified in commensal microbes in various environmental and host ecosystems (1-8). The illustration of commensals as facilitators for AR gene dissemination (4), and the correlation of antibiotic usage in animals with increased AR in human microbiota (5,6) suggest the importance of commensals in mediating the dissemination of AR genes. The isolation of AR genes in foodborne pathogens and opportunistic pathogens from retail products exemplified the potential contribution of the food chain in transmitting ART pathogens to humans (7,8). But pathogens only count for a very small percentage in the microflora and they don't serve as a significant source in AR transmission. Our recent study showed that foodborne commensal bacteria , on the other hand, can carry as much as 10^7 CFU of ART bacteria per gram of food, suggesting food can be an important avenue in the dissemination of AR genes. To properly evaluate and investigate the AR risk associated with the food chain, a broader spectrum of foods need to be examined. The objective of this study is to assess the prevalence of AR in selected food items as well as the functionality, transferability and stability of the AR genes.

METHODS



RESULTS & DISCUSSION

Table 1. AR gene carrier identification

Food sample	Tet^r gene(S)	Identified carrier(s)
raw shrimp-S1	*tetS*	*Carnobacterium sp.*
raw shrimp-S2	*tetS, tetM*	*Carnobacterium sp.* (*tetS*)
cooked shrimp-S3	*tetS, tetM*	*Carnobacterium sp.* (*tetS*)
cooked shrimp-S4	*tetS*	*Carnobacterium sp.* (*tetS*)
Sushi-F3	*tetS, tetM*	*Carnobacterium sp.*
raw shrimp-S12	*tetS, tetM*	*Carnobacterium sp.* (tetS), *Brochothrix sp.* (tetM)
raw shrimp-S15	*tetM*	*Carnobacterium sp.*
Cheese-M7	*tetM, tetL*	*Enterococcus sp.* (*tetM & tetL*)

Detection of Tet^r Gene in ART Isolates

Representative ART isolates from each sample were analyzed for the presence of representative Tet^r genes including conventional PCR by specific primers. The *tetS* and *tetM* genes were detected from both cooked and raw shrimp samples as well as sushi samples while *tetM* and *tetL* genes were both found from a cheese sample (Table 1).

Identification of AR Gene Carriers

Representative positive isolates were further identified using 16S rRNA gene sequence analysis. Several isolates of *Carnobacterium sp.* carrying *tetS* or *tetM*, one isolate of *Brochothrix sp.* carrying *tetM* and two isolates of *Enterococcus sp.* carrying both *tetM* and *tetL* were identified (Table 1).

Natural Transformation of *Streptococcus mutans*

The *tetS* gene from cooked shrimp isolates *Carnobacterium sp.* S3TG251 and S12BTG16, *tetM* gene from raw shrimp isolates *Carnobacterium sp.* S2TG12 and *Brochothrix sp.*S12BTG32, sushi isolate *Carnobacterium sp.* F3BTG36 and cheese isolates *Enterococcus sp.* M7-M2 and M7-BTG14 were successfully transmitted to human oral residential bacterial isolate *Streptococcus mutans* UA159 and led to acquired resistance in the progenies. The *tetL* gene in *Enterococcus sp.* M7-M2 and M7-BTG14 was transferred along with *tetM* to the recipient strain. PCR amplification confirmed the presence of the Tet^r genes in the *S. mutans* transformants.

Determination of the Genetic Location of the Tet^r Genes

Isolates showed different plasmid profiles by plasmid extraction (Fig. 1). Results from the Southern blotting analysis showed different locations of the *tetS and tetM* genes (data not shown). Plasmids carrying *tetS or tetM* gene from *Carnobacterium sp.* isolates and a 20-kb plasmid carrying both *tetM and tetL* from *Enterococcus sp.* were identified.

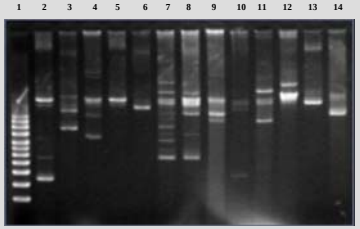


Fig. 1. Plasmid profiles of representative isolates. Lane 1: Supercoil Ladder (Biorad); Lane 2:S1TG21; Lane 3: S2TG12; Lane 4: S3TG251; Lane 5: S3TG27; Lane 6: S4TG342; Lane 7: S11BTG18; Lane 8: S12BTG16; Lane 9: S12BTG32; Lane 10: S13BTG16; Lane 11: S15BTG14; Lane 12: S12BTG32; Lane 13: S13BTG16; Lane 14:S15BTG14.

Plasmid Stability Test and Partial Sequence Analysis

The 20-kb plasmid is very stable without the tetracycline and at the absence or presence of 10 ug/ml acridine orange after 30 subsequent culturing. The data suggest that the corresponding antibiotic is not required in the stable transmission of the plasmid, and other mechanism(s) is involved in the maintenance of the AR genes. Partial DNA sequence analysis showed that *tetS*-encoding plasmid from *Carnobacterium sp.* S3TG251 contains the resolvase gene next to *tetS*, and the 20-kb *Enterococcus sp.* plasmid contains genes *tetM*, *tetL*, as well as the plasmid mobilization gene *mob*.

CONCLUSIONS

Results from this study showed that Tet^r bacteria are prevalent in both raw and ready-to-eat food items. The *tetS*, *tetL* and *tetM* genes were found in the food isolates, and identified AR-gene carriers included *Carnobacterium sp.*, *Brochothrix sp.*, and *Enterococcus sp.*. The AR genes from selected food isolates were successfully transmitted to *S. mutans* via natural transformation, confirming their mobility and functionality. The data suggest that food intake can be an important avenue transmitting AR genes to the general public, therefore like is partially responsible for the observed AR in human oral and gut microflora (9,10). Our results further suggest that antibiotics may not be the essential element in the maintenance and transmission of these AR genes, as believed in the past. Thus the agriculture and food industry need to develop novel control strategies to minimize the transmission of AR to human through the food chain.

REFERENCES

1. Nandi, S., Maurer, J. J., Hofacre, C. and Summers, A.O. (2004) Gram-positive bacteria are a major reservoir of Class 1 antibiotic resistance integrons in poultry litter. *Proc. Natl. Acad. Sci.* USA **101**, 7118-7122.
2. Salyers, A. A., Gupta, A. and Wang,Y. (2004) Human intestinal bacteria as reservoirs for antibiotic resistance genes. Trends Microbiol. **12**, 412-416.
3. Lancaster, H., Ready, D., Mullany, P., Spratt, D., Bedi, R. and Wilson, M. (2003) Prevalence and identification of tetracycline-resistant oral bacteria in children not receiving antibiotic therapy. FEMS Microbiol Lett. **228**, 99-104.
4. Luo, H., Wan, K. and Wang, H.H. (2005) A high frequency conjugation system facilitated biofilm formation and pAMβ1 transmission in *Lactococcus lactis*. Appl. Environ. Microbiol. **71**, 2970-2978
5. Levy, S. B., FitzGerald, G. B. and Macone, A. B. (1976) Changes in intestinal flora of farm personnel after introduction of a tetracycline-supplemented feed on a farm. N. Engl. J. Med. **295**, 583-588.
6. Smith, D. L., Harris, A. D., Johnson, J.A., Silbergeld, E. K. and Morris, J. G. (2002) Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. Proc. Natl. Acad. Sci. USA. **99**, 6434-6439.
7. Johnston, L. M. and Jaykus, L. A. (2004) Antimicrobial resistance of *Enterococcus* species isolated from produce. Appl. Environ. Microbiol. **70**, 3133-3137.
8. Wang, H., Manuzon, M., Lehman, M., Wan, K., Luo, Wittum, T.E., Yousef, A., Bakaletz., L. (2006) Food commensal microbes as a potentially important avenue in transmitting antibiotic resistance genes. FEMS Microbiol. Lett. **254**, 226-231.
9. Villedieu, A., Diaz-Torres, M.L., Hunt, N., McNab, R., Spratt, D.A., Wilson, M. and P. Mullany. (2003). Prevalence of tetracycline resistance gene in oral bacteria. Antimicrobial Agents and Chemotherapy **47**, 878-882
10. Gueimonde, M., S. Salminen, E Isolauri (2006) Presence of specific antibiotic (tet) resistance genes in infant faecal microbiota. FEMS Immunol Med Microbiol **48**, 21-25.

ACKNOWLEDGMENTS

The study is supported by OSU start-up fund, OARDC projects OHOA1265, OHOA 1315, OHO00208H for H. Wang.

*Corresponding author: Dr. Hua H. Wang, 110 parker Food Science & Technology, The Ohio State University, Columbus, OH, 43210-1007
E-mail: wang.707@osu.edu; Tel. (614) 292-0579.

Department of Food Science and Technology, The Ohio State University, Columbus, OH 43210